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MEASUREMENT OF TRABECULAR BONE VOLUME: A COMPARATIVE STUDY OF HISTOMORPHOMETRY AND COMPUTED TOMOGRAPHY

Jonathan L. Jacobs

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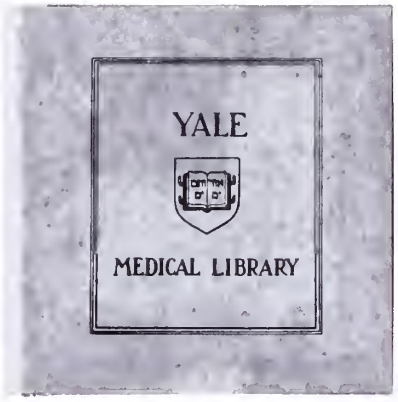
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Measurement of Trabecular Bone Volume: A Comparative Study of Histomorphometry and Computed Tomography

by Jonathan L. Jacobs

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirement for the degree of Doctor of Medicine

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Introduction

Although much of bone is inorganic matrix, it should not be considered a devitalized tissue. The various types of bone cells actively and continually serve the organism; performing at least two major functions. First,they are responsible for the formation and remodelling of the matrix which provides mechanical support. Moreover, the matrix and the cells that lie within it play essential roles in the maintenance of homeostasis of Ca^{++}, HPO_4^{--}, H^+, and Mg^{++}.[27] Through incompletely understood mechanisms involving bone formation and resorption, bone cells help regulate the amount of these substances available to the body or locked within the ionic reservoir formed by the bony skeleton.

Either or both of these functions can be deranged in the group of disorders classified as metabolic bone diseases. Alterations in the biochemical regulating functions of bone cells are often characterized by changes in serum and urine levels of the aforementioned ions, parathyroid hormone, calcitonin, bone cell enzymes, and collagen breakdown products [27]

Before the introduction of laboratory methods to detect the biochemical hallmarks of these diseases, clini-

cians were often unaware of their presence until they noted the gross changes in bony architecture that can accompany advanced illness. Plain radiographs were not useful for early detection of disease. In those disorders causing osteoporosis, plain radiographic techniques could not detect changes of less than 25-35% of vertebral bone mass.[26]

Quantitative studies of changes in bony structures were difficult in living patients. Even at autopsy, evaluation of disease was often crude. One pathologist recalls that as late as 1954, the method used to judge whether a cadaver evidenced osteomalacia was an attempt to crush a vertebra between thumb and forefinger. Osteoporosis and the effect of aging on bone were investigated using Archimede's principle and the ability of a specimen to displace water or millet seed.[1]

Sampling of diseased tissue in patients became possible with the introduction of trephines for ileal biopsies.[29] The ilia offer three major advantages as biopsy sites. They are accesible to physicians using only minor surgical techniques. Also, they form part of the axial skeleton; the portion of the bony structure of most clinical interest. Finally, the ilia are composed of cortical and trabecular bone in proportions which approximate those for the entire

skeleton.[19]

Analysis of cortical bone is important in that the cellular activity per unit surface of cortical bone is greater than the same parameter in trabecular bone. However, trabecular bone has a much larger surface to volume ratio than cortical bone. In fact, this difference is so large that changes in bone volume and remodelling rates are greater in the cancellous than in the cortical portions of bone.[19] These histologic facts are often manifest clinically.[17] Changes in the femora and vertebrae frequently herald the onset of disease because these structures contain a large amount of trabecular bone, and are major weight bearing structures. They become weak because they suffer a great loss in terms of percent bone volume.

Using a variety of histomorphometric techniques a sample of bone can be analyzed to determine the amount of bone, the mineralization rate, and osteocyte, osteoblast, and osteoclast activity. One important parameter is the trabecular bone volume.[6] As noted above, patients are often symptomatic from the complications of the pathologic loss of trabecular bone. After studying such patients as well as normal subjects, investigators have established that trabecular bone volume varies according to the person's

age and sex.[2,9,21,22,31] The results of one series are presented in Table 1.

Table 1. Mean Values (±S.D.) of Iliac Trabecular Bone Volume (T.B.V.) in 236 Controls

Age	15-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99
Males									
Number of Cases	8	38	26	33	27	8	8	2	
Mean Age	17.7	24.4	35.5	44.9	54.3	64.0	75.0	81.5	
T.B.V.	21.9	22.3	19.7	19.2	18.8	16.8	17.8	18.0	
	±2.8	±4.8	±4.8	±2.8	±4.8	±2.8	±4.9	±4.0	
Females									
Number of Cases	4	21	10	9	12	8	11	8	3
Mean Age	16.7	24.0	34.5	44.5	53.7	64.3	74.8	83.0	92.0
T.B.V.	24.5	23.5	23.1	21.6	19.9	13.4	15.9	12.5	16.0
	±2.9	±5.8	±4.3	±2.8	±3.6	±3.3	±2.9	±2.0	±1.6

(From: Meunier P., Courpron P. in Bone Morphometry, Edited by Z.F.G. Jaworski, University of Ottawa Press, pp. 100-105)

In males cancellous bone rarefaction appears to follow an approximately linear progression with age. Females, on the other hand, lose trabecular bone slowly until age 50. From 50-70 the bone loss is more rapid. After 70, the

rate of loss again diminishes. Courpron and Meunier also established that all patients regardless of age or sex were prone to vertebral collapse if their ileal trabecular bone volume was less than 11%(±3).[9]

It should be noted that the parameter trabecular bone volume may reflect changes in cortical bone. That is, the ratio of volume of bone within the trabecular cavity to the volume of the cavity itself is affected by endosteal resorption of compact bone. This phenomenon can lead to overestimation of trabecular bone volume.

Despite the theoretical utility of following trabecular bone volume and other histologic measurements in patients with metabolic bone disease, a number of concerns have been voiced about diagnostic ileal biopsies. One disadvantage of any biopsy technique is the morbidity of the minor surgery involved. Although the morbidity associated with bone biopsies is low, numerous non-invasive techniques have been proposed to study bony tissues *in vivo*

Another spur to the development of alternative methods for assessing metabolic bone disease is the difficulty of interpreting biopsy results. There is, for example, a controversy concerning the representativeness of the iliac sample[15 23] As noted above, Courpron and Meunier were able to relate biopsy measurements to vertebral collapse. These

authors argue in a number of papers that there is a good correlation between loss of bone at the biopsy site and clinical osteoporosis.[21,22] Vost, however, showed that in patients reaching autopsy there was only a gross relationship between the amount of bone in samples from biopsy sites and in samples from vertebral bodies.[35] Anderson, Dunnill, and Whitehead also assert that ilial biopsies are not necessarily representative.[12]

Perhaps part of the problem is related to the variability of the bony architecture within the ilia. One recent paper described biopsies in which there was as much as 50% variation in samples taken from sites only 1-2 centimeters from each other.[3] Other investigators argue against the importance of variability in the interpretation of biopsy measurements. One experienced laboratory reports a difference of less than 10% in trabecular bone volume in adjacent samples from normal and diseased patients.

Despite such claims, it is clear that in some cases even repeated analyses of the same biopsy material can yield different trabecular bone volumes. Bordier, for example, noted that measured bone volumes from two sections of the same sample could vary up to 25%.[5] Methods of quantification, degree of magnification, and inter-observer variability have all been cited as factors which add uncertainty to histomorphometric appraisal of bone volume.

Another factor which probably contributes to the apparent variability in biopsy analysis is the structure of the bone itself. Singh has shown that cancellous bone is not a uniform structure. Instead, there are at least nine histologic subtypes of trabecular bone.[33] The distribution of these subtypes has not been totally defined; but may affect the baseline bone density as well as the apparent progression of disease in different areas of trabecular bone.

Researchers have proposed a number of alternatives to biopsy for the assessment of trabecular bone volume. Before describing some of these methods, it should be noted that even if successful, none of these techniques could replace the biopsy. A bone sample can be analyzed to yield information about cell activity and mineralization rate; as well as bone volume. None of the new methods can claim such versatility or utility. However, it is hoped that answers can be sought non-invasively when the crucial clinical question involves bone mass or degree of mineralization.

One relatively simple method for the assessment of osteoporosis requires only a plain radiograph of the hip. The trabeculae in the upper end of the femur are arranged in five major groups. In progressive osteoporosis, those

groups least required to bear the stress disappear first. Thus, by noting the number and position of trabecular groups in a radiograph of the hip, the relative degree of osteoporosis can be determined.[11,33]

Another method which utilizes plain radiographs is photodensitometry. A wedge of known density is placed alongside one of the patients phalanges, and the two are radiographed. A computerized densitometer is then used to calculate the bone mineral density. At a given degree of mineralization, this latter value theoretically varies directly with bone mass. Unfortunately, this procedure is technically difficult, and cannot analyze trabecular bone separately from the cortical bone surrounding it.[17, 20]

A more popular method, photon absorbitometry has a similar drawback. In this procedure, I^{125} is used as a monochromatic gamma ray source. The beam is aimed at the radius, and the amount of photon energy absorbed by that bone is measured. The degree of energy attenuation is directly related to the bone mass traversed by the beam. However, as in photodensitometry, the energy absorbed is due to both cortical and trabecular bone.[8,17,34] Investigators have attempted to minimize this problem by bombarding two portions of the radius. The distal radius is mostly trabecular bone; while more proximal portions contain almost ex-

clusively cortex.[23] The radius was chosen for scanning because of convenience and because changes in radial bone mass appears to reflect the loss of bone from the skeleton. However, the correlation between bone mass in th radius and the spine is not absolute.[34]

The body's calcium and, by extrapolation, bone content has also been successfully measured using neutron activation analysis. All or part of a patient is exposed to a neutron source. Like the studies mentioned above, this method relies on the difference between the energy of particles entering the skeleton and the energy of particles leaving it. Although very accurate and reproducible, neutron activation requires expensive equiptment and high levels of radioactive exposure. Also, like photodensitometry, trabecular bone cannot be analyzed separately from cortical bone.

The advent of computerized tomography hascreated a revolution in diagnostic radiology. This method's ability to detect intracranial masses by recognizing minor differences in tissue density has been well established. An increasing number of uses are being discovered for the diagnosis of lesions in other parts of the body. During the past few years, researchers have investigated the use of computerized tomography to assess bone volume <u>in vivo</u>

The CT scanner has the capability of reproducing ana-

tomic cross sections. This is accomplished by radiographically examining the patient at a large number of different angles. Based on the results of these scans, a computer can reconstruct an image of the subject using a complex mathematical algorithm. In the mathematical represntation of the subject which precedes the image formation, the various elements of the cross section are converted into a two dimensional matrix of numbers assigned values from -1000 to +1000. These values are directly related to the density of the matter traversed by the x-ray beams. Thus, in the ideally calibrated system air has a value of -1000 and water has a value of 0.

Like all of the non-invasive techniques mentioned so far, computerized tomography ultimately relies on the attenuation of the energy of the beam passing through an object. Computerized tomogarphy, however, offers at least two theoretical advantages over other methods. Photodensitometry and photon absorbitometry are both limited to the examination of peripheral structures. In the future, it will be hypothetically possible to direct the CT scanner at components of the axial skeleton.[14] Moreover, with its ability to discriminate between adjacent areas of differing density the CT scanner should, for the first time, allow for the differential analysis of trabecular and cortical bone.

A number of studies have established the potential of computerized tomography for bone mineral analysis. In one experiment, the calcium content of vertebrae obtained at autopsy was shown to be linearly related to the CT numbers.[7] In another study, the correlation between CT values and calcium content in dog bones was shown to be .9975. In that same paper computerized tomography was shown to be as accurate and precise as photon absorbitometry.[26]

Although these in vitro trials are encouraging, they do not contend with the difficulties of using the scanner on patients. These include subject repositioning, patient motion, and beam hardening artifact. Another potential problem, already alluded to in reference to the biopsy, is the heterogeneity of trabecular bone. In the cadaver vertebrae study, slight repositioning of the two bones being longitudinally and transversely scanned yielded surprisingly different results (Table 2).

In a paper describing CT examination of cadaver arms from both normal and demineralized patients, the potential problem was illustrated in a different way. CT values were predictably lower in the demineralized than in the normal subjects. Of interest is the fact that the demineralized specimens showed a marked increase in the variability of values within the individual bones.[22]

Table 2. Normalized ACTA Numbers of L2 and L3 of a Specimen in Ten Scans*

Scan Number	(ACTA Number of Bone minus Water)/Water	
	L2	L3
1(T)	.18	.08
2(L)	.10	.12
3(L)	.15	.09
4(L)	.10	.15
5(L)	.15	.08
6(L)	.14	.11
7(T)	.21	.15
8(L)	.22	.13
9(L)	.20	.12
10(L)	.17	.16

*T=transverse; L=longitudinal

(From:Bradley J.G., Huang H.K., Ledley R.S., Radiology, 126:103, 1978

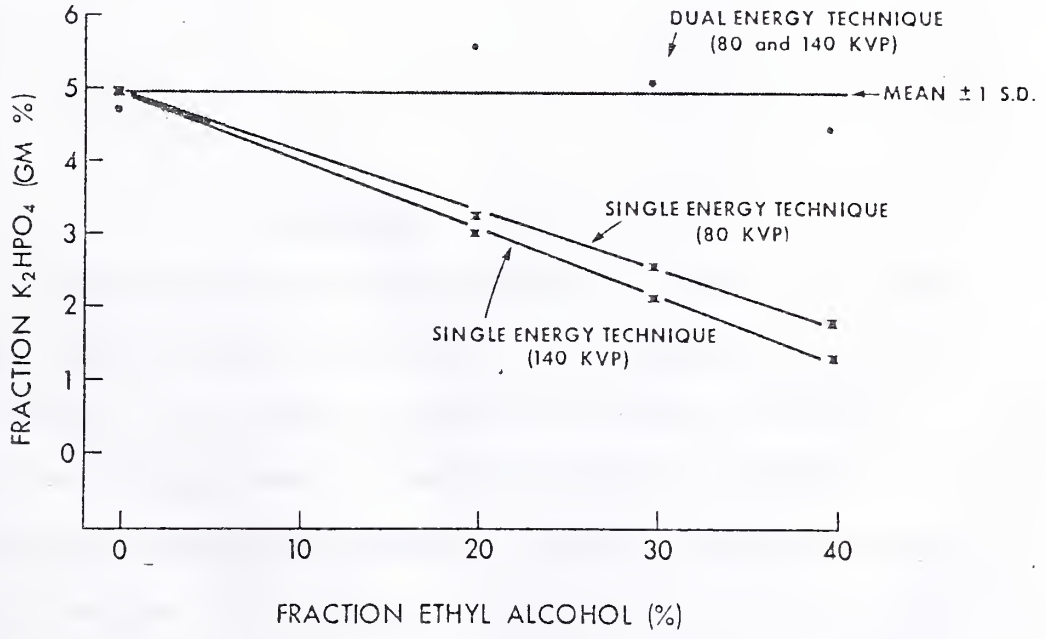
One probable factor responsible for the greater variation of density within diseased bone is the increased amount of fat in the medullary cavity.[14] In one experiment, the effect of different amounts of fat within bones on CT scans was simulated using phantoms of known densities. In particular, K_2PO_4 was used to simulate bone mineral; and ethanol to simulate fat. Since the amount of energy absorbed by a substance depends on the energy of the scanning beam as well as the density of the object scanned, the investigators sought to solve for the bone mineral content alone using two different energy scanners. The degree of energy attenuation produced by the simulated bone mineral varied considerably at different scanning energies

But the simulated fat's attenuation of energy changed very little in different preliminary scans. Thus, using simultaneous equatiions, the researchers showed that they could solve for simulated bone content no matter how much simulated fat was added.[14]

The drawback to the dual energy scanning method is illustrated in Figure 1. Although the dual energy scanning

Figure 1. ACCURACY FOR MINERAL DETERMINATION

Measured K_2HPO_4 Fraction (5gm.%) vs. Alcohol Admixtures (0-40%) For Dual Energy and Single Energy Techniques



(From: Genant H K., Boyd D., Invest. Radiol , 12:545, 1977)

method is accurate, it is not as precise as single energy

computerized tomography. The authors concluded that for use in the same patient, the precision of the single energy scan made it the preferable technique [14]

In the present experiment we have attempted to demonstrate a correlation between trabecular bone volume as determined by single energy computerized tomography and as determined by histomorphometric analysis of bone biopsies. We hope thereby to test for the first time the utility of the CT scan as an *in vivo* method for assessing that type of bone which shows the earliest changes in metabolic bone disease

Materials and Methods

Thirty patients were included in the study. Their ages, which ranged from 18-75, and diagnoses are shown in Table 3 It should be noted that those subjects who do not carry diagnoses of bone disease were suspected of having bony changes secondary to their primary problem at the time the study was performed. These patients were chosen from an original group of fifty patients. Criteria for exclusion were focal rather than generalized disease, and technically inadequate bone biopsy or CT scan. The pa-

tient with Paget's disease included in the study was known not to have focal pelvic involvement. Although some patients were found to have normal trabecular bone volumes, all patients had a history of bone disease.

Table 3.

Patient	Age	Diagnosis
1	53	primary hyperparathyroidism
2	57	primary hyperparathyroidism
3	26	osteoporosis
4	50	primary biliary cirrhosis
5	43	primary hyperparathyroidism
6	18	hypophosphatemic rickets
7	59	primary hyperparathyroidism
8	60	hypophosphatemic osteoporosis
9	57	hypophosphatemic osteoporosis
10	23	chronic renal failure
11	61	primary hyperparathyroidism
12	63	primary hyperparathyroidism
13	54	primary hyperparathyroidism
14	40	hypophosphatemic osteoporosis
15	38	pseudo hypoparathyroidism
16	59	primary hyperparathyroidism
17	59	osteomalacia
18	50	primary biliary cirrhosis
19	53	malabsorption
20	61	primary hyperparathyroidism
21	24	osteoporosis
22	56	renal tubular acidosis
23	54	osteoporosis
24	68	phenobarbitol associated osteomalacia
25	36	nephrolithiasis
26	68	primary hyperparathyroidism
27	34	primary hyperparathyroidism
28	38	primary hyperparathyroidism
29	75	primary hyperparathyroidism
30	71	Paget's disease

Each patient had undergone bone biopsy and CT scan within seven weeks of each other. The biopsies were per-

formed by one of three physicians

The computerized tomography was performed using a Pfizer 0200 FS (140 KVP) scanner, in the manner already described by Orphanoudakis, et al.[17,25] To minimize patient motion artifact, the patient's hand and forearm were placed in a plexiglas glove, which was surrounded by water in an attached plexiglas cylinder. The glove-cylinder assembly was then inserted into the scanner; kept in position by a special holder at the back of the machine This assembly allowed the patient's forearm to be moved forward and backward for proper positioning; but almost eliminated movement once the scanning site was selected.

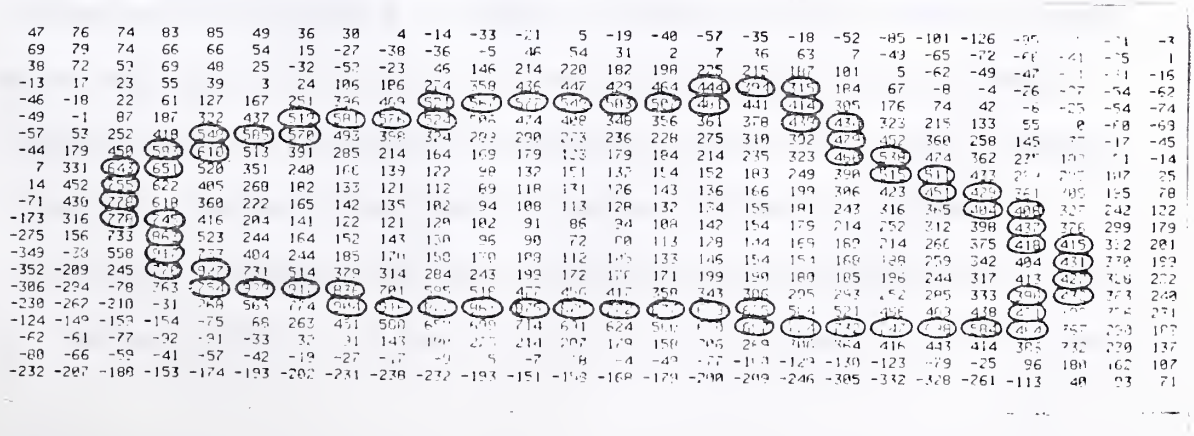
Surrounding the cylinder was a plexiglass ring containing K_2PO_4 phantoms of different concentrations These standards were used to calibrate the machine between scans.

It had previously been determined that the distance between the forearm bones, as well as their cross sectional areas, could be used to repeatedly identify a given radial scanning site with excellent precision. It is this capability of repeatedly studying almost identical sections of bone that would seem to make the scanner ideal for longitudinal investigations In the present study, the radiologist selected scanning sites approximately 1-4 cm from the distal articulation of the radius; a region containing a large percentage of cancellous bone. A number of scans were

performed on each patient. For this study, those scans taken at sites closest to a radial-ulnar distance of 10.0 were selected.

For each patient, the scanner was calibrated, and then instructed to concentrate on the area of the subject occupied by the radius. The numerical matrix resulting from the scan of the radial cross section was then analyzed as follows. Using a mathematical algorithm that identifies maximum local CT numbers, the scanner constructed a boundary of numbers with large values that theoretically corresponds to the anatomic area containing the densest cortical bone. This assumption is based on the fact that CT numbers are inversely proportional to the degree of attenuation of the energy beam as it traverses tissue. Thus, numbers should increase with the examination of fat, soft tissue, trabecular bone, and cortical bone successively. An example of a boundary constructed within a mathematical matrix representing the radius is shown below.

Figure 2.



Using this boundary as a reference line, concentric layers, each 1 mm. wide, were analyzed to yield the average CT numbers of matter within that layer (pixel). The outermost layer was labelled 0, with each successive subcortical layer assigned a higher number. The radial-ulnar distance, total cross sectional area and number of points within each layer were also recorded for every scan.

The biopsies were performed, in most cases, under local anesthesia. In a few instances, the biopsies were taken at the time of parathyroid exploration under general anesthesia Six mm. Bordier trephines were used to obtain horizontal sections of the iliac crest that contained the two cortices. The bone samples were dehydrated using a succession of graded alcohols. They were embedded, undecalcified, in methylmethacrylate. Longitudinal sections 4 microns thick were made using a Jung K microtome or Jung Autocut microtome These sections were then stained according to the Von Kossa technique.

Stained samples were then photographed at a magnification of 16X using a Reichart microscope and Polaroid camera and film The cortices were then identified on each photographed section and, beginning with the outer boundary of the cortex, the samples were divided by parallel lines into sections 16 mm. wide (corresponding to an actual tissue width of 1 mm.). As with the scans, the outermost

1 mm. sections were labelled 0. Successive subcortical divisions were assigned higher numbers. A sample photograph is shown in figure 3.

Figure 3.

According to the Delesse principle, bone volume is directly related to the area occupied by bone. To calculate the bony area ineach biopsy photograph and its subdivisions, I used a Manual Optical Planimeter, MOP (Zeiss, Germany). This machine consists of a planimeter grid, a tracing element, and a computer. Photographs were placed on the planimeter, and the bony perimeters in each section were traced. The MOP automatically calculates the area of the grid under the photograph, delineated by the tracing element. Trabecular bone volume was calculated according to

the following equation:

$$\frac{\text{Area of section occupied by bone}}{\text{Total area of section}} \times 100$$

In making these calculations, the corresponding sections from either side of the biopsy were taken together. That is, the numerator included the area of both sections 0 (or 1, 2, etc.) occupied by bone; while the denominator was the added total areas of those two sections.

Results

Comparisons were made between trabecular bone volume as determined by histomorphometric analysis of iliac bone biopsies and computerized tomography of the radius. Correlation was tested using regression analysis and Spearman's rank analysis. For each comparison, a line was generated, and r^2, r, the standard error of estimation and Spearman's rho were calculated.

Figure 4 shows the relationship between the average trabecular bone volume for the biopsy except the 0 layer, and the average CT number for the radial scan except the 0 pixel. The outer portions were excluded to allow assess-

ment of trabecular bone volume without the cortices. The slope of the line was 8.99 with an intercept of 58.89. Standard error of estimation was 83.75. r^2 was 0.46, and r was 0.68. The F ratio of 23.92 was significant at the .01 level. A Spearman's rho of .6253 was significant at the .002 level.

Regional values within the biopsies and scans were also compared. Specifically, we were interested in the regions closest to the center of the biopsies and radii. The biopsy and scan from the same patient was often of unequal width. That is, it was not unusual to have a biopsy with five subsections and a corresponding CT scan with eight pixels. To surmount this inequality, and insure inclusion of comparable portions of bone in the analysis, we made five separate comparisons of the values describing the most central regions of each bone.

First, the trabecular bone volume of the most central layer of each biopsy was compared to the average CT number of the most central pixel of the corresponding scan. Figure 5 shows this comparison. 110 has been added to each CT value to avoid negative numbers and facilitate statistical analysis. The slope of the line is 6, and the y intercept is 37.59. r^2 was 0.33, giving an r of 0.57. The standard error of estimation was 66.53. An F ratio of

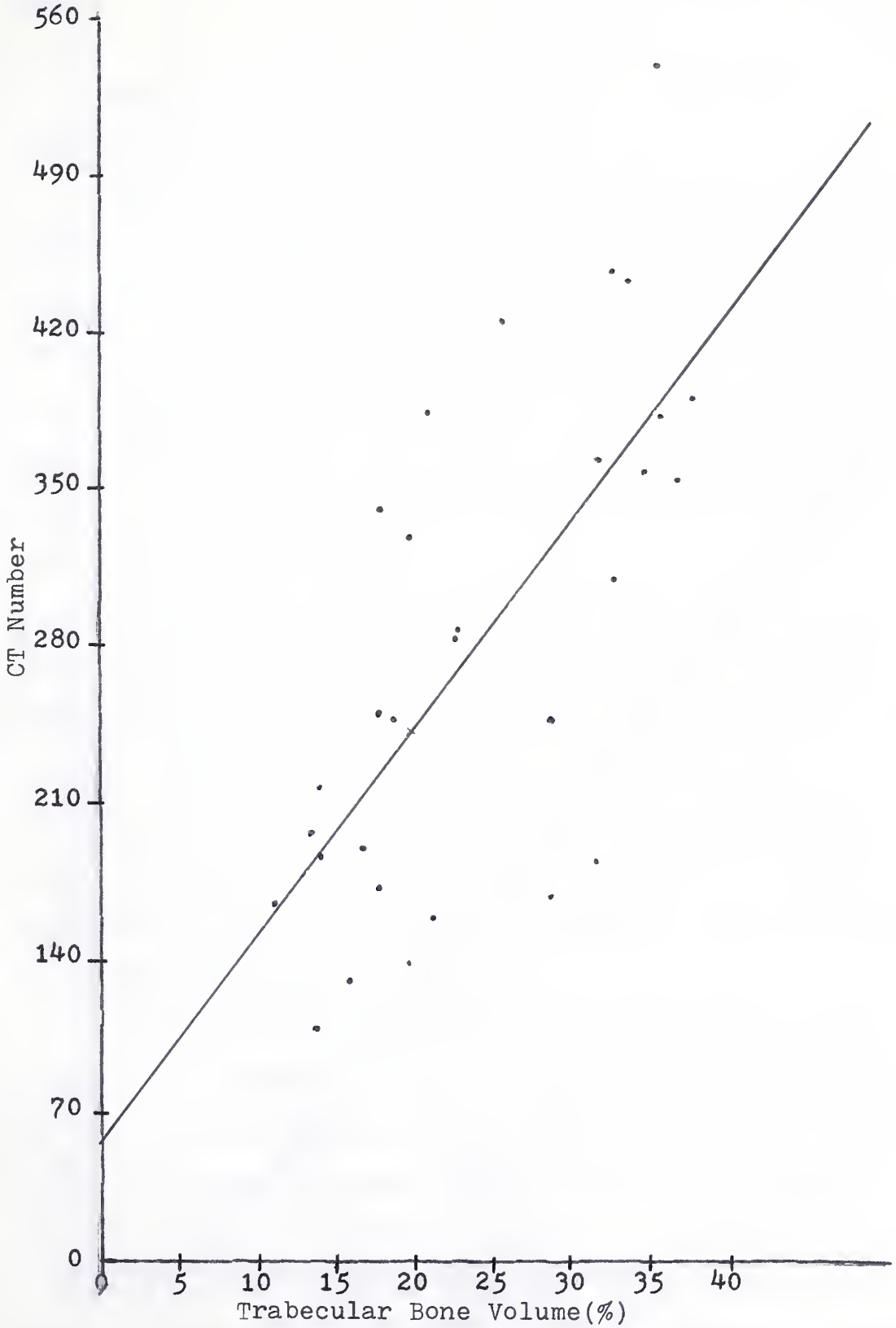


Figure 4. Plot of average trabecular bone volume versus average CT number

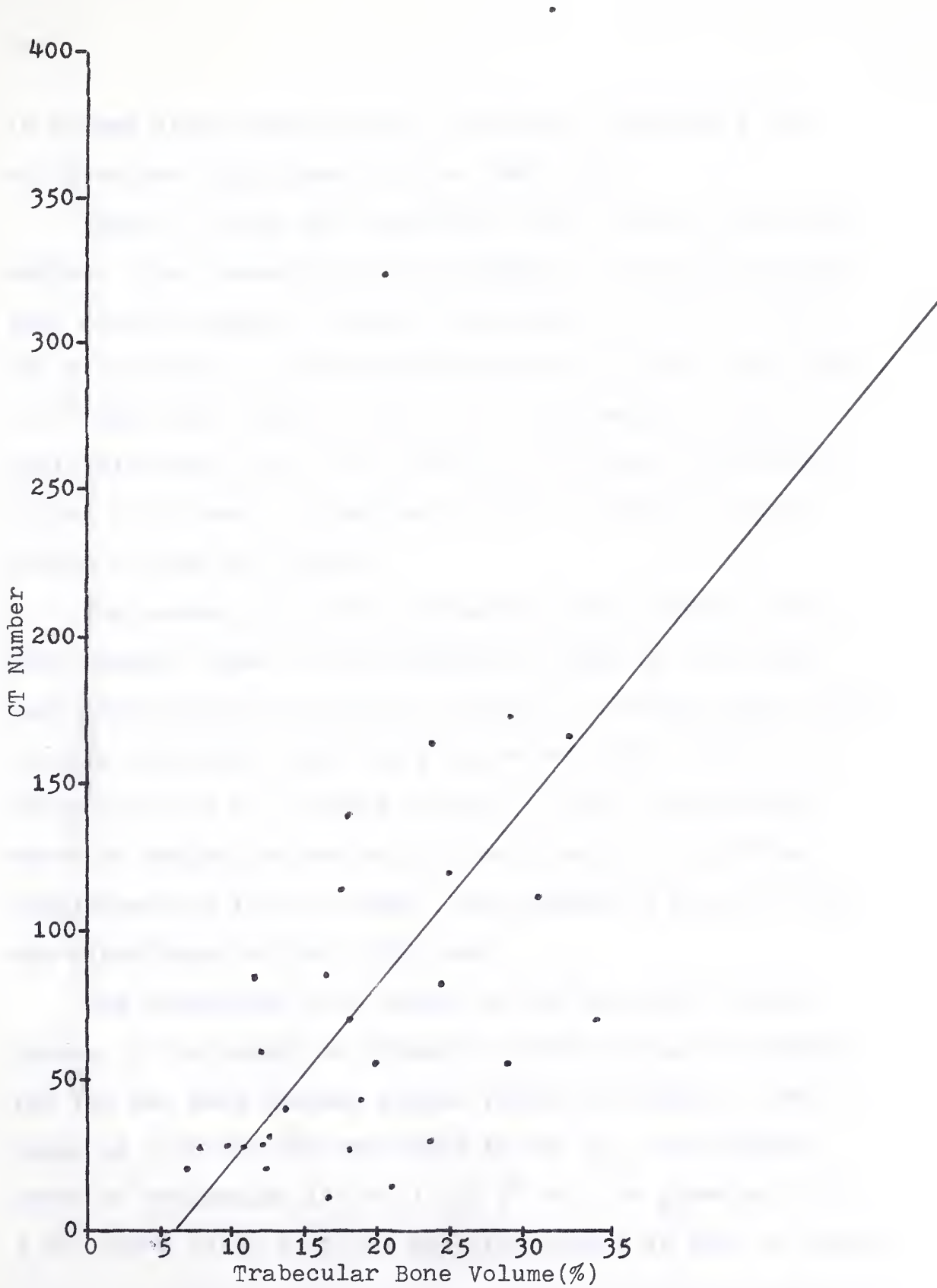


Figure 5. Plot of trabecular bone volume of most central biopsy layer versus CT value of most central pixel (+110).

14.05 was significant at the 0.01 level. Spearman's rho of 5666 was significant at the .002 level.

Figure 6 shows the trabecular bone volume of the most central layer compared to the average CT value for the two most central pixels. 100 has been added to each CT value. The slope was 6.29, with an intercept of -29.89. The value of r^2 was 0.32, with an r of 0.57. The standard error of estimation was 71.98. An F ratio of 13.20 was significant at the 0.01 level. A Spearman's rho of .5632 was significant at the .002 level.

The comparison of the trabecular bone volume of the most central layer to the average CT value of the innermost three pixels is shown in figure 7. 100 was again added to each CT number. The line's slope was 8.38, with an intercept of -17.6. r^2 was 0.34 with r 0.58. The standard error of estimation was 92.30. The F ratio of 13.28 was significant at the .01 level. The Spearman's rho of .5649 was significant at the .002 level.

The tarbecular bone volume of the two most central layers of the biopsy is compared to the average CT number for the two most central pixels (#100) in figure 8. The slope is 7.02 and the intercept is -51.31. The standard error of estimation is 72.23. An r^2 of 0.32 gives an r of 0.57. The F ratio of 12.92 was significant at the .01 level.

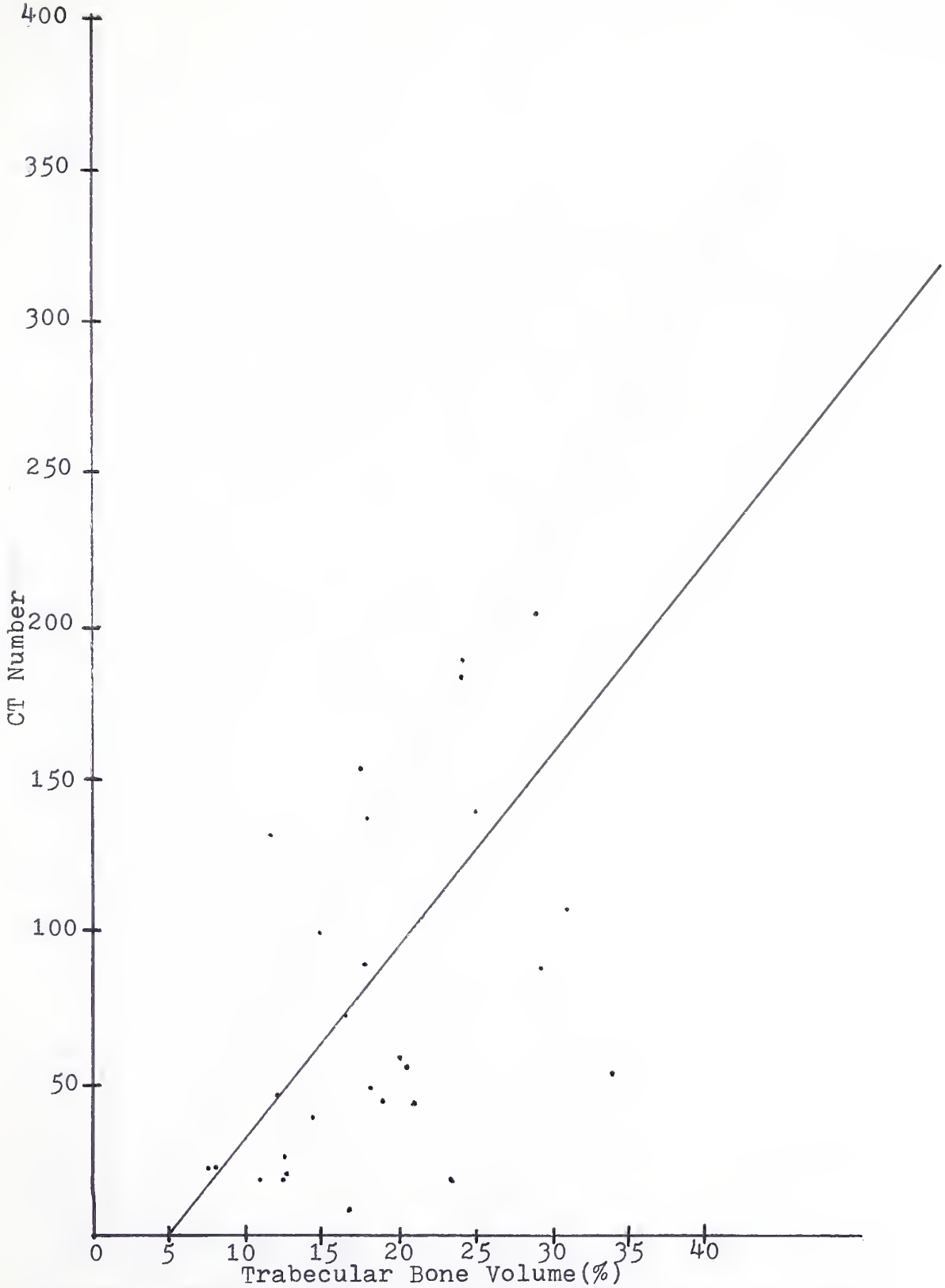


Figure 6 Plot of trabecular bone volume of most central biopsy layer versus average CT value of two most central pixels (+100)

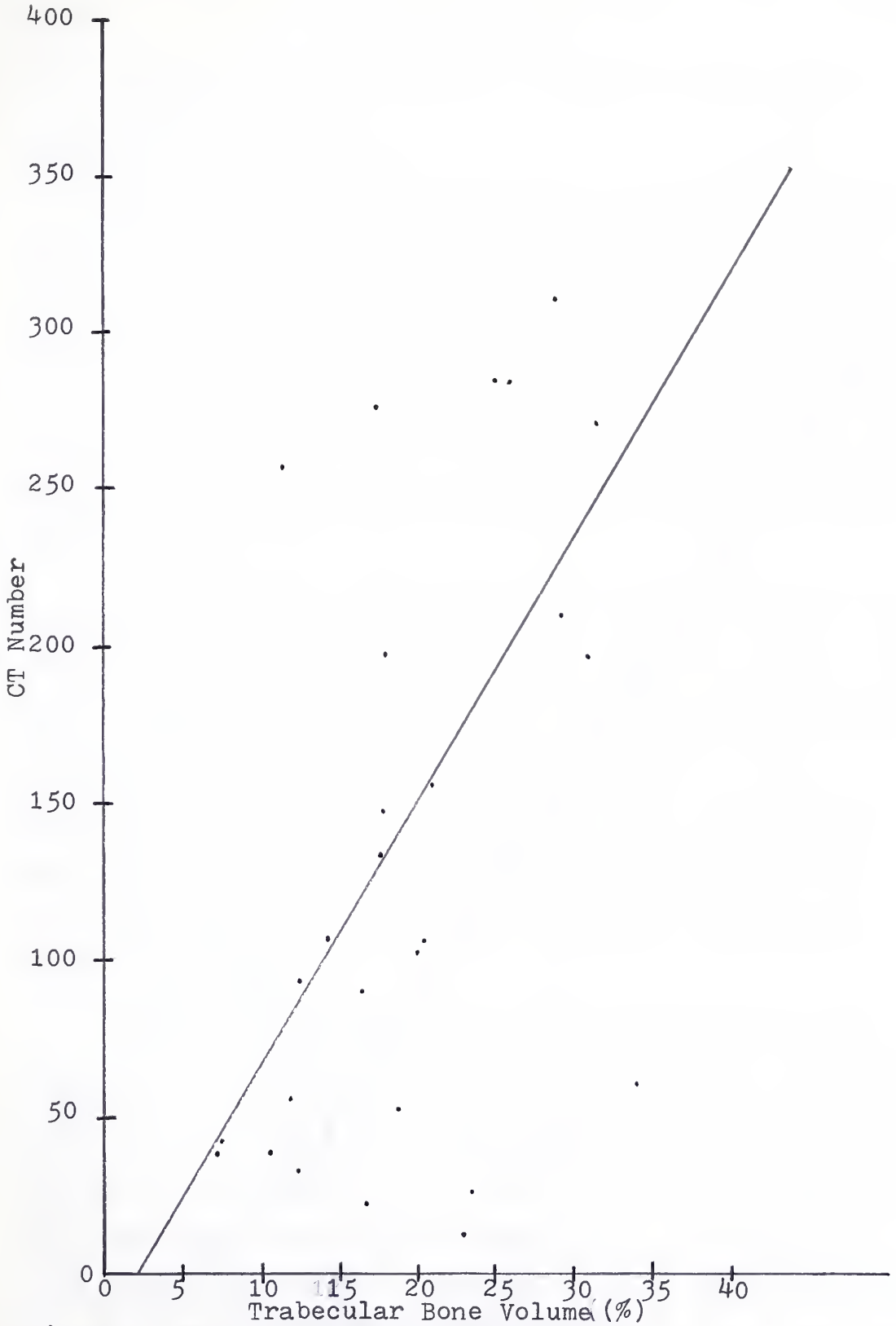


Figure 7. Plot of trabecular bone volume of most central biopsy layer versus average CT value of three most central pixels (+100)

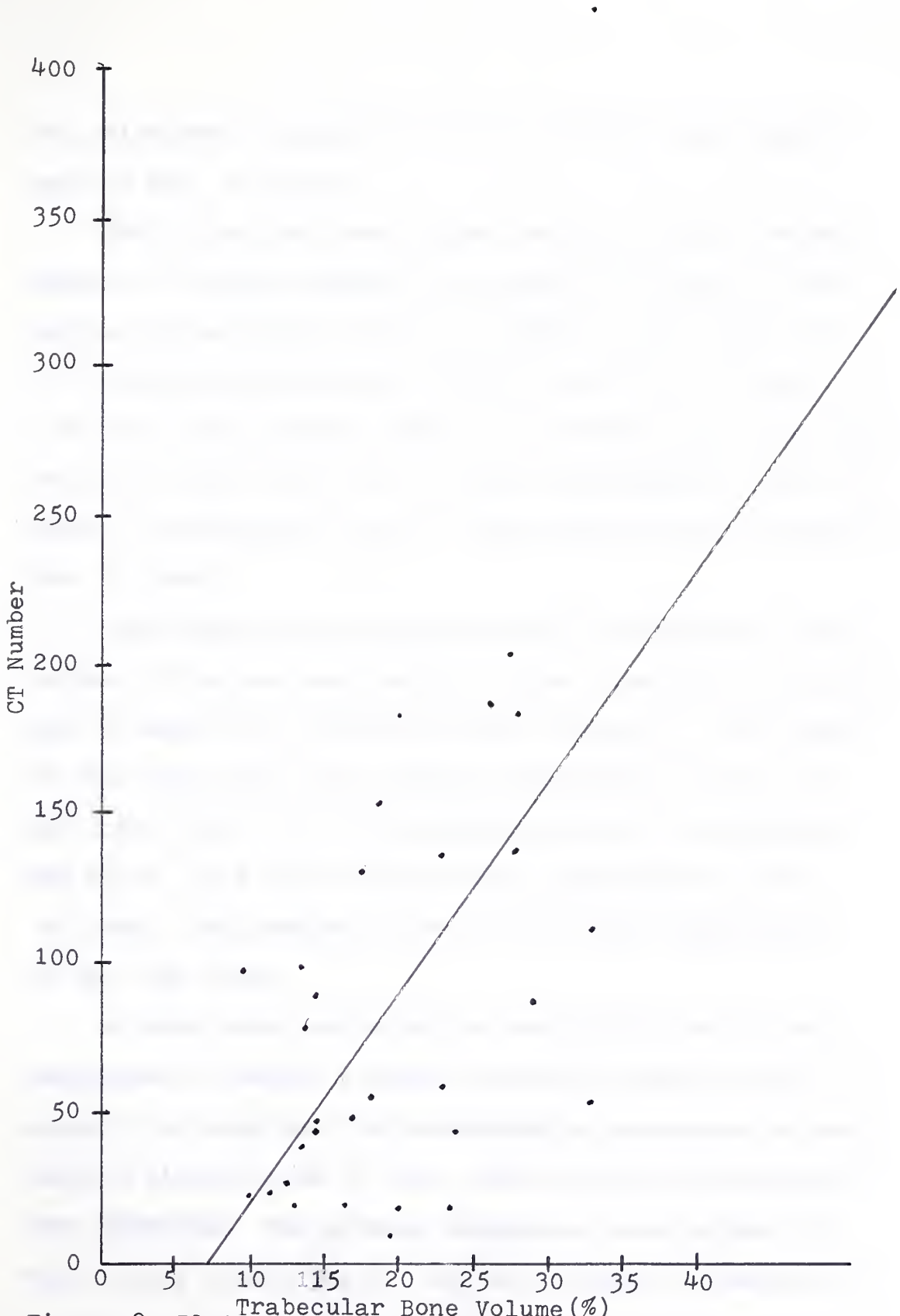


Figure 8. Plot of trabecular bone volume of two most central biopsy layer versus average CT value of two most central pixels(+100)

The calculated Spearman's rho of .5368 was also significant at the .01 level.

The trabecular bone volume for the two most central layers is plotted against the average CT numbers of the central three pixels (+100) in figure 9. The slope was 9.45 and the intercept was -49.27. An r^2 of 0.35 gave an r of 0.59. The standard error of estimation was calculated at 91.25. The F ratio of 14.18 was significant at the .01 level. The Spearman's rho of .5286 was also significant at the .01 level.

The final direct comparison was the trabecular bone volume of the two most central biopsy layers and the average CT number for the entire scan (figure 10). The slope of this line was 10.92, with an intercept of 56.35. r^2 was 0.45; with r 0.67. The standard error of estimation was 84.74. An F ratio of 22.72 was significant at the .01 level. The Spearman's rho of .6477 was significant at the .002 level.

We were also interested in the ability of the two techniques to detect regional variation within bones. To compare the scan and histomorphometric assessment of non-uniform distribution of bone, the following calculations were performed. The average trabecular bone volume for each biopsy (excluding 0), and the average CT number for

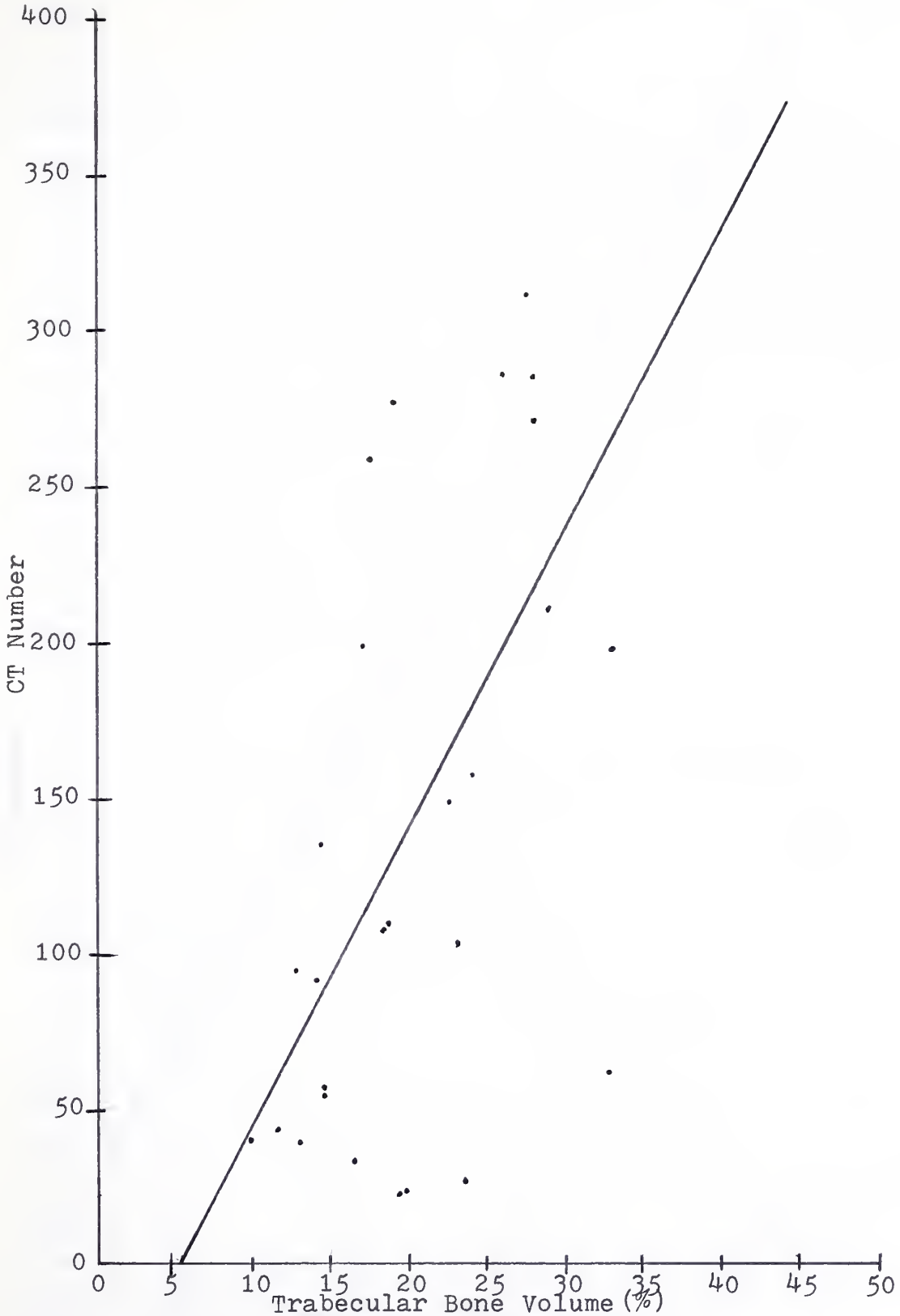


Figure 9, Plot of trabecular bone volume of two most central biopsy layers versus average CT value of three most central pixels(+100)

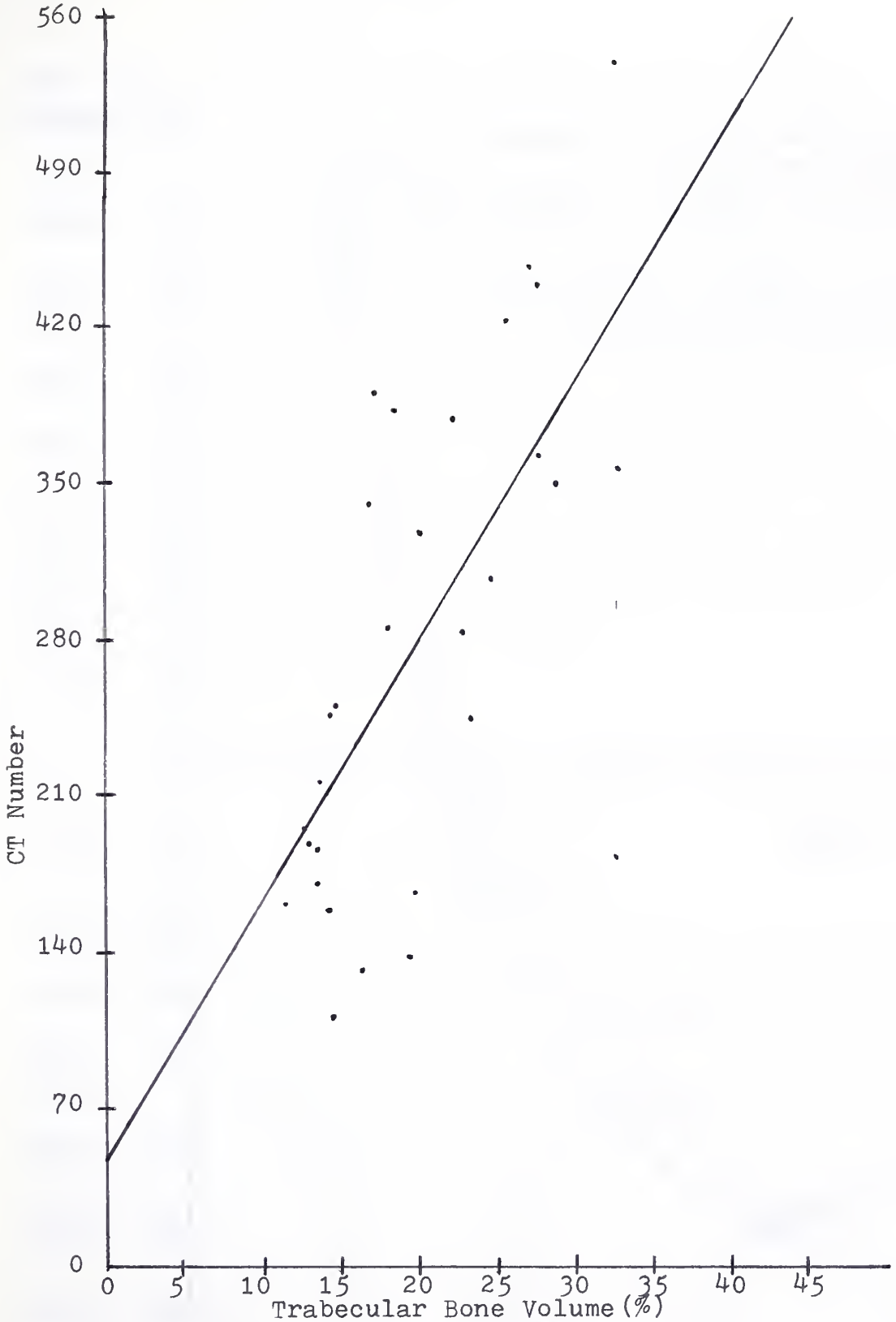


Figure 10 Plot of trabecular bone volume of two most central biopsy layers versus average CT number.

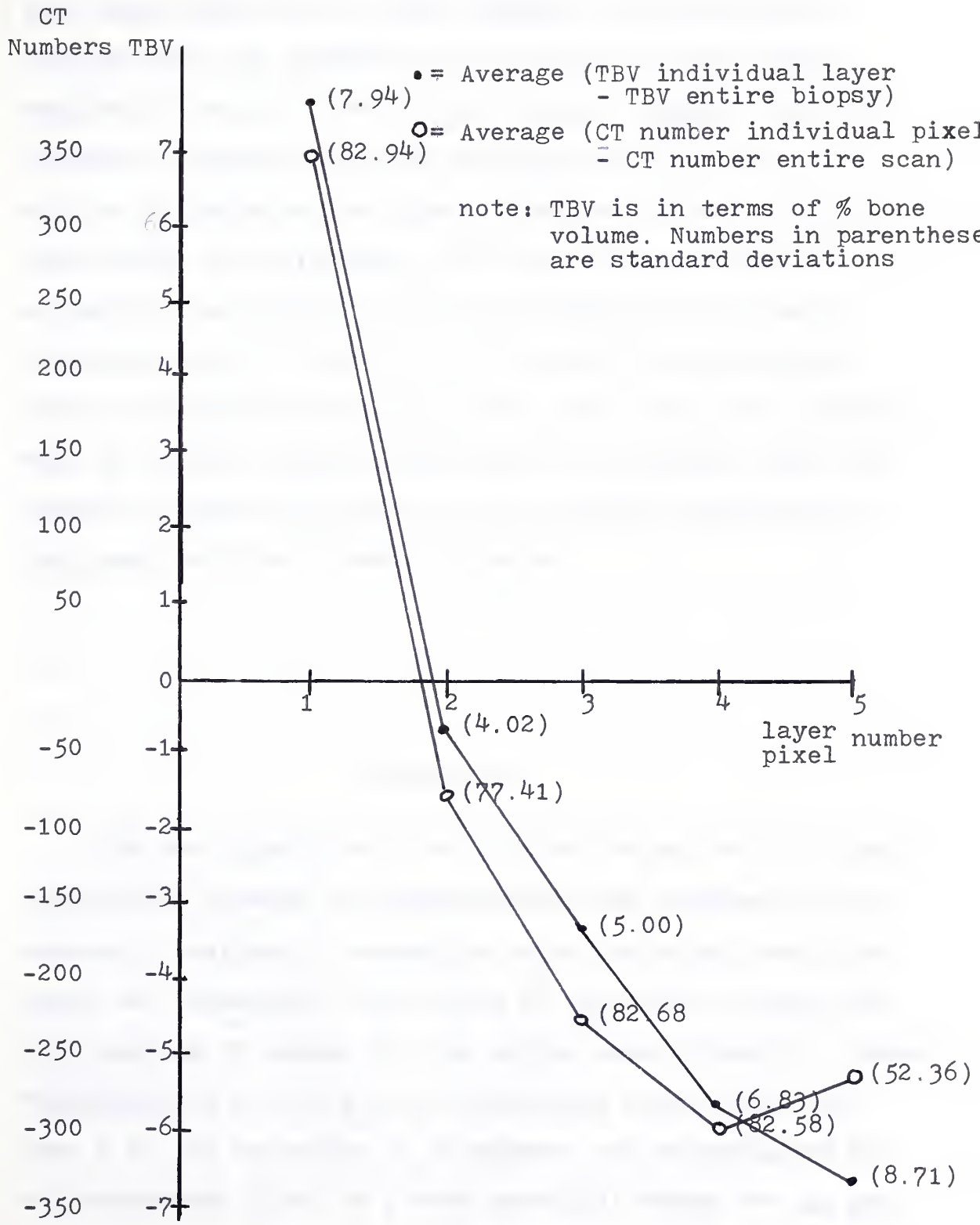

CT
Numbers TBV
• = Average (TBV individual layer - TBV entire biopsy)
O= Average (CT number individual pixel - CT number entire scan)
note: TBV is in terms of % bone volume. Numbers in parentheses are standard deviations
350
300
250
200
150
100
50
0
-50
-100
-150
-200
-250
-300
-350
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
1
2
3
4
5
layer number
pixel
(7.94)
(82.94)
(4.02)
(77.41)
(5.00)
(82.68)
(6.83)
(82.58)
(52.36)
(8.71)

Figure 11.

each scan (excluding 0) were obtained. These were subtracted from the trabecular bone volumes of each layer and the CT number of each pixel for each patient. The differences obtained were then averaged for each slice (i.e. all the differences for layer 1) and each pixel. Finally, the average of differences for each slice was plotted alongside the average of the differences for its corresponding pixel in figure 11. The numbers in parentheses are the standard deviations of the data. Since the biopsies were of varying lengths, the number of patients whose difference values contributed to the averages decreased as the layer and pixel number increased.

Discussion

The most significant correlation for any of the direct comparisons between histomorphometric and computerized tomographic analyses is associated with the relationship between the trabecular bone volume of the entire biopsy and the average CT number for the entire scan (figure 4). Nevertheless, even for this plot, statistics reveal that less than $\frac{1}{2}$ of the variation in CT numbers can be explained by the regression line. In a more practical sense, we can see

that it would be difficult to predict trabecular bone volume from a given CT value. For the same CT value can apparently correspond to a wide range of measured bone volumes At the same time it must be recognized that non- parametric analysis shows a significant correlation for the bone volumes and CT numbers. There is a relationship between the values obtained by the two techniques; but it is a rough one.

This result is by no means surprising. As noted earlier there are a host of variables that can significantly alter the bone volumes of samples taken only a few millimeters from each other. In the current study we compared different parts of the skeleton. Added to all these other factors is a patient population spanning 60 years and suffering more than a dozen different diseases

Yet out of this mixture of variables has emerged the relationship illustrated in figure 11. Before discusssing this encouraging result, it is worthwhile to examine the group of regional comparisons.

There were two reasons for focusing our attention on the most central portion of the biopsies and scans. First, an important difference between the two techniques was the method of distinguishing cortex. The outer limit of cortical bone was identified in the biopsies; with

subsequent divisions made referent to these boundaries. No attempt was made to delineate the inner cortical boundaries. The CT scanner, on the other hand, was programmed to identify the largest CT numbers and construct a limit It was assumed that the circle thus constructed would lie within the densest bone - that is, the center of each cortex. Therefore, starting from the outside boundaries used by each technique would constitute starting from different referents. Starting from the center of the biopsies and scans, and proceeding outwards, would standardize the two techniques for comparison.

Moreover, clinical interest is often centered on patients with low trabecular bone volume. As will be discussed below, the biopsies were discovered to have non- homogeneous bone content. Usually, it was the center of the specimen that had the lowest regional density. Therefore, by concentrating on these regions, we could compare the two methods using bone volumes of the greatest clinical interest.

As was noted earlier, five separate comparisons were made to adjust for the varying widths of the biopsies and scans. In each case the non-parametric analysis shows significance. However, regression analysis, as well as simple observation show considerable variation around the regres-

sion line That variance is lessened when more of the bone closer to the cortices is included in the comparison (figures 4 and 10). One factor which undoubtedly contributes to the poor correlation between the CT and histomorphometric values at the center of bone is fat. The CT values for many of the central pixels were negative. Had the scanning beams encountered only mineral within that space, the numbers would not have been so low Instead, we must assume that the most central parts of many of the scanned bones contained varying mixtures of mineral and soft tissue; a factor which does not affect histomorphometric measurement. If the relationship illustrated in figure 1 also describes what occurs in vivo, then the single energy scanner's accuracy would decrease with increasing fat content.

Theoretically, one manifestation of intramedullary fat should be lower CT values with increasing age. We have not followed any one subject long enough to adequately test this hypothesis. However, there does not seem to be any consistent relationship in the graphs between age and CT number.

It is difficult to conclude anything about the characteristics of patients with different diseases because of the small subject population. In all the graphs, the patients with primary biliary cirrhosis appear to have

high CT values corresponding to high trabecular bone volumes The patients with primary hyperparathyroidism show a poor correlation between CT and biopsy results; especially in the regional comparisons. Part of this appearance is probably attributable to sampling artifact. Patients with primary hyperparathyroidism constitute half of the sample examined. Still, in each of the plots there are several patients with this disease who have anomolously low CT numbers. The reason for this is unclear.

Future investigations of the ability of single energy computerized tomography to assess trabecular bone volume should include studies in which the biopsy and scan examine the same bone. Scans of cadaver materials which could subsequently be subjected to ash weight analysis might be very useful

Given the proven precision of the single energy technique, longitudinal studies in patients should also be undertaken.

The potential for longitudinal studies is also suggested by the remarkable relationship shown in figure 11 Direct comparisons between measured trabecular bone volume and CT values (figures 4-10) demonstrated only rough linear correlations. Fat and other variables that prob-

ably affected these results have already been discussed Another factor which influenced the direct comparisons was variation in the distribution of bone. We observed in the course of regional analyses of the biopsies that trabecular bone volume was rarely uniform within a sample. For instance, tissue in the immediate subcortical regions was usually denser than tissue in the central areas. This is reflected in the increasingly negative differences between regional trabecular bone volume and overall trabecular bone volume shown in figure 11. One implication of this variability is that the average trabecular bone volume routinely measured could depend on length of biopsy sample and amount of tissue from different regions, as well as stage of disease.

This factor was to a great degree eliminated in the comparison illustrated in figure 11. Starting from baselines self-defined for both histomorphometry and computerized tomography showed the intra-technique variations to be strikingly similar despite rough inter-technique correlations. In fact, the data would seem to suggest that if a common baseline could be derived, single energy computerized tomography could assess trabecular bone volume as well as histomorphometric analysis of bone biopsy.

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